

**IOWA DEPARTMENT OF NATURAL RESOURCES
ENVIRONMENTAL SERVICES DIVISION
FORM 30 - NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM PERMIT APPLICATION**

PART C – TOXICITY TESTING DATA

POTWs meeting one or more of the following criteria must provide the results of pass/ fail 48-hour acute whole effluent toxicity (WET) tests for each of the facility's discharge points: 1) POTWs with a design flow rate greater than or equal to 1.0 mgd; 2) POTWs with a pretreatment program (or those that are required to have one under 40 CFR Part 403); or 3) POTWs required by the permit writer to submit the results of WET testing.

- Submit the results of any whole effluent toxicity tests from the past four years. If a whole effluent toxicity test conducted during the past four years revealed toxicity, provide any information on the cause of the toxicity or any results of a toxicity reduction evaluation.
- **If you have already submitted any of the information requested in Part C, you need not submit it again.** Rather, provide the information requested in question 2 for previously submitted information.

The facility is not required to perform any additional WET testing as part of this form, unless it is specifically requested by the permit writer.

If the permit writer requests that the facility submit the results of whole effluent toxicity testing, based on total dissolved solids (TDS) or chloride sample results from the facility that indicate a potential for toxicity in the receiving stream, the facility must provide the information requested in question 5. If this information is not requested, question 5 can be left blank.

If no toxicity testing is required, do not complete Part C. Refer to the Application Overview for directions on which additional parts to complete.

FACILITY NAME:

PERMIT NUMBER:

1. REQUIRED TESTS

Indicate the number of whole effluent toxicity tests conducted in the past four years.
 ___ acute ___ chronic

2. SUMMARY OF SUBMITTED TEST INFORMATION

If you have submitted biomonitoring test information, or information regarding the cause of toxicity, within the past four years, provide the approximate dates the information was submitted to the Department and a brief summary of the results (see instructions).

Dates submitted:

Summary of results: (see instructions)

3. TOXICITY REDUCTION EVALUATION

Is the treatment works involved in a Toxicity Reduction Evaluation (TRE)?

☐ Yes ☐ No

If yes, describe:

If the facility has performed pass/fail 48-hour acute toxicity tests in the past four years **for which results have not been submitted to the Department**, proceed to question 4 (next page). If the permit writer requests a pass/fail 48-hour acute toxicity test, report the results in question 4. Make additional copies of page 2 of Part C as needed. Please supply this application to the testing laboratory as needed. If the facility has performed any chronic toxicity tests in the past four years **for which results have not been submitted to the Department**, attach the results to this form and submit them with the application packet.

FACILITY NAME:	PERMIT NUMBER:																																																																																																																																											
4. PASS/ FAIL 48-HOUR ACUTE TEST DATA	<div style="border: 1px solid black; padding: 5px;"> a. Facility Data <table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td style="padding: 2px;">i. Outfall Number (refer to Part A, question 14 a.):</td></tr> <tr><td style="padding: 2px;">ii. Sampling Location If the facility uses chlorination, indicate whether the sample was taken before or after the dechlorination facilities: Before: <input type="checkbox"/> After: <input type="checkbox"/> Not Applicable: <input type="checkbox"/></td></tr> <tr><td style="padding: 2px;">iii. Diluted Effluent Sample Ratio (from current permit or permit writer):</td></tr> <tr><td style="padding: 2px;">iv. Lab Sample Number:</td></tr> <tr><td style="padding: 2px;">v. Date of Sample Collection:</td></tr> <tr><td style="padding: 2px;">vi. Date Received (by lab) :</td></tr> <tr><td style="padding: 2px;">vii. Temperature upon receipt (in degrees C):</td></tr> </table> </div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"> b. Organism Data <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 2px;"> i. <u>Pimephales Promelas</u> age: _____ Reference Toxicant: _____ Reference LC₅₀: _____ Date: _____ </td> <td style="width: 50%; padding: 2px;"> ii. <u>Ceriodaphnia Dubia</u> age: _____ Reference Toxicant: _____ Reference LC₅₀: _____ Date: _____ </td> </tr> </table> </div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"> c. Sample Data <table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td style="padding: 2px;">i. Temperature (in Degrees C):</td></tr> <tr> <td style="padding: 2px;">ii. Dissolved Oxygen</td> <td style="padding: 2px;">Initial: _____ mg/L</td> <td style="padding: 2px;">Final: _____ mg/L</td> </tr> <tr> <td style="padding: 2px;">iii. pH</td> <td style="padding: 2px;">Initial: _____ S.U.</td> <td style="padding: 2px;">Final: _____ S.U.</td> </tr> <tr> <td style="padding: 2px;">iv. Total Residual Chlorine</td> <td style="padding: 2px;">Initial: _____ mg/L</td> <td style="padding: 2px;">Final: _____ mg/L</td> </tr> <tr> <td style="padding: 2px;">v. Specific Conductance</td> <td style="padding: 2px;">Initial: _____ umhos/cm</td> <td style="padding: 2px;">Final: _____ umhos/cm</td> </tr> <tr><td style="padding: 2px;">vi. Ammonia Nitrogen (as N): _____ mg/L</td></tr> <tr><td style="padding: 2px;">vii. Total Ammonia (as NH₃): _____ mg/L</td></tr> <tr><td style="padding: 2px;">viii. Unionized Ammonia (calculated as NH₃): _____ mg/L</td></tr> <tr><td style="padding: 2px;">ix. Total Dissolved Solids: _____ mg/L</td></tr> <tr><td style="padding: 2px;">x. Chloride: _____ mg/L</td></tr> </table> </div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"> d. Mortality (Bioscreen) Data <table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td colspan="14" style="padding: 2px;">Laboratory Name:</td></tr> <tr> <td colspan="7" style="padding: 2px;">Test begun date:</td> <td colspan="7" style="padding: 2px;">Test end date:</td> </tr> <tr> <td colspan="8" style="padding: 2px; text-align: center;">Pimephales Promelas Mortality</td> <td colspan="6" style="padding: 2px; text-align: center;">Ceriodaphnia Dubia Mortality</td> </tr> <tr> <td colspan="8" style="padding: 2px;">Number dead or affected/ Number tested</td> <td colspan="6" style="padding: 2px;">Number dead or affected / Number tested</td> </tr> <tr> <td style="padding: 2px;">Conc</td> <td style="padding: 2px;">BK 1</td> <td style="padding: 2px;">BK 2</td> <td style="padding: 2px;">BK 3</td> <td style="padding: 2px;">BK 4</td> <td style="padding: 2px;">P</td> <td style="padding: 2px;">F</td> <td style="padding: 2px;"></td> <td style="padding: 2px;">BK 1</td> <td style="padding: 2px;">BK 2</td> <td style="padding: 2px;">BK 3</td> <td style="padding: 2px;">BK 4</td> <td style="padding: 2px;">P</td> <td style="padding: 2px;">F</td> </tr> <tr><td style="padding: 2px;">Ctrl</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr> <tr><td style="padding: 2px;">100%</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr> <tr><td style="padding: 2px;">Diluted</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr> </table> </div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"> Effluent Toxicity testing Result: Pass: <input type="checkbox"/> Fail: <input type="checkbox"/> Date Reported: _____ Comments: _____ </div>	i. Outfall Number (refer to Part A, question 14 a.):	ii. Sampling Location If the facility uses chlorination, indicate whether the sample was taken before or after the dechlorination facilities: Before: <input type="checkbox"/> After: <input type="checkbox"/> Not Applicable: <input type="checkbox"/>	iii. Diluted Effluent Sample Ratio (from current permit or permit writer):	iv. Lab Sample Number:	v. Date of Sample Collection:	vi. Date Received (by lab) :	vii. Temperature upon receipt (in degrees C):	i. <u>Pimephales Promelas</u> age: _____ Reference Toxicant: _____ Reference LC ₅₀ : _____ Date: _____	ii. <u>Ceriodaphnia Dubia</u> age: _____ Reference Toxicant: _____ Reference LC ₅₀ : _____ Date: _____	i. Temperature (in Degrees C):	ii. Dissolved Oxygen	Initial: _____ mg/L	Final: _____ mg/L	iii. pH	Initial: _____ S.U.	Final: _____ S.U.	iv. Total Residual Chlorine	Initial: _____ mg/L	Final: _____ mg/L	v. Specific Conductance	Initial: _____ umhos/cm	Final: _____ umhos/cm	vi. Ammonia Nitrogen (as N): _____ mg/L	vii. Total Ammonia (as NH ₃): _____ mg/L	viii. Unionized Ammonia (calculated as NH ₃): _____ mg/L	ix. Total Dissolved Solids: _____ mg/L	x. Chloride: _____ mg/L	Laboratory Name:														Test begun date:							Test end date:							Pimephales Promelas Mortality								Ceriodaphnia Dubia Mortality						Number dead or affected/ Number tested								Number dead or affected / Number tested						Conc	BK 1	BK 2	BK 3	BK 4	P	F		BK 1	BK 2	BK 3	BK 4	P	F	Ctrl														100%														Diluted													
i. Outfall Number (refer to Part A, question 14 a.):																																																																																																																																												
ii. Sampling Location If the facility uses chlorination, indicate whether the sample was taken before or after the dechlorination facilities: Before: <input type="checkbox"/> After: <input type="checkbox"/> Not Applicable: <input type="checkbox"/>																																																																																																																																												
iii. Diluted Effluent Sample Ratio (from current permit or permit writer):																																																																																																																																												
iv. Lab Sample Number:																																																																																																																																												
v. Date of Sample Collection:																																																																																																																																												
vi. Date Received (by lab) :																																																																																																																																												
vii. Temperature upon receipt (in degrees C):																																																																																																																																												
i. <u>Pimephales Promelas</u> age: _____ Reference Toxicant: _____ Reference LC ₅₀ : _____ Date: _____	ii. <u>Ceriodaphnia Dubia</u> age: _____ Reference Toxicant: _____ Reference LC ₅₀ : _____ Date: _____																																																																																																																																											
i. Temperature (in Degrees C):																																																																																																																																												
ii. Dissolved Oxygen	Initial: _____ mg/L	Final: _____ mg/L																																																																																																																																										
iii. pH	Initial: _____ S.U.	Final: _____ S.U.																																																																																																																																										
iv. Total Residual Chlorine	Initial: _____ mg/L	Final: _____ mg/L																																																																																																																																										
v. Specific Conductance	Initial: _____ umhos/cm	Final: _____ umhos/cm																																																																																																																																										
vi. Ammonia Nitrogen (as N): _____ mg/L																																																																																																																																												
vii. Total Ammonia (as NH ₃): _____ mg/L																																																																																																																																												
viii. Unionized Ammonia (calculated as NH ₃): _____ mg/L																																																																																																																																												
ix. Total Dissolved Solids: _____ mg/L																																																																																																																																												
x. Chloride: _____ mg/L																																																																																																																																												
Laboratory Name:																																																																																																																																												
Test begun date:							Test end date:																																																																																																																																					
Pimephales Promelas Mortality								Ceriodaphnia Dubia Mortality																																																																																																																																				
Number dead or affected/ Number tested								Number dead or affected / Number tested																																																																																																																																				
Conc	BK 1	BK 2	BK 3	BK 4	P	F		BK 1	BK 2	BK 3	BK 4	P	F																																																																																																																															
Ctrl																																																																																																																																												
100%																																																																																																																																												
Diluted																																																																																																																																												
Attach any chronic toxicity test results to this form, and submit them with the application packet.																																																																																																																																												

If the TDS or chloride data submitted by the facility (Part A, question 17), indicates that the levels of TDS in the effluent may cause toxicity in the receiving stream, the facility may be required to perform chronic and/or acute whole effluent toxicity tests. If such testing is required, the facility must provide the information below in addition to submitting the laboratory report. **If the permit writer does not request this testing, question 5 should be left blank.**

5. ACUTE AND CHRONIC TEST RESULTS

a. Acute Test Results

Parameter	Result - %	Result – mg/L
LC ₅₀	%	mg/L
NOAEC	%	mg/L
95% C.I.	%	mg/L
Control percent survival	%	NA
Other (describe)	%	mg/L

b. Chronic Test Results

Parameter	Result - %	Result – mg/L
NOAEC	%	mg/L
IC ₂₅	%	mg/L
95% C.I.	%	mg/L
Control percent survival	%	NA
Other (describe)	%	mg/L

c. Ion Concentrations in Sample (100% effluent)

Ion	Concentration
Bicarbonate (HCO ₃)	mg/L
Calcium (Ca)	mg/L
Chloride (Cl ⁻)	mg/L
Carbonate (CO ₃)	mg/L
Total Iron (Fe)	mg/L
Magnesium (Mg)	mg/L
Potassium (K)	mg/L
Sodium (Na)	mg/L
Sulfate (SO ₄)	mg/L
Nitrate (NO ₃) + Nitrite (NO ₂)	mg/L
TDS	mg/L

END OF PART C

FORM 30, PART C - INSTRUCTIONS

Treatment works meeting one or more of the following criteria must complete Part C (Toxicity Testing Data):

- Treatment works with a design flow rate greater than or equal to one mgd; or
- Treatment works with an approved pretreatment program (as well as those required to have one under 40 CFR Part 403); or
- Treatment works otherwise required by the permit writer to submit the results of whole effluent toxicity (WET) testing.

Test results provided in Part C must be based on the testing procedures specified in 567-Chapter 63.4 "Effluent Toxicity Testing Required in Permits". The data provided in Part C must be based on tests performed within four years prior to completing this application. Additional copies of Part C may be used in submitting the required information. Do not include information on combined sewer overflows in Part C.

Applicants only need to submit data that have not previously been submitted to the DNR. Thus, if test data have already been submitted (within the last four years) in accordance with an issued NPDES permit, the treatment works need not fill out the information requested in question 4. A permittee having no significant toxicity in the effluent over the past year and who has submitted all toxicity test results through the end of the calendar quarter preceding the time of permit application would need to supply no additional toxicity testing data as part of this application. Instead, the applicant should complete question 2 and question 3. Question 2 requests a summary of toxicity test information already submitted. If the applicant conducted a whole effluent toxicity test during the past four years that revealed toxicity, then provide any information available on the cause of the toxicity or any results of a toxicity reduction evaluation (TRE), if one was conducted. Question 3 requests a description of any TREs. (See below for more detailed instructions on completing questions 2, 3, and 4).

If the facility is required by the permit writer to perform acute and/or chronic whole effluent toxicity testing based on the results of effluent TDS samples, question 5 must be filled out. Question 5 may be left blank if the facility has not been required to perform WET testing based on TDS sample results. See below for more detailed instructions on completing question 5.

1. Required Tests

Provide the total number of acute and chronic whole effluent toxicity tests conducted in the past four years. An "acute" toxicity test is one in which the effect is observed in 96 hours or less. A "chronic" toxicity test continues for a relatively long period of time, often one-tenth the life span of the organism or more.

2. Summary of Submitted Test Information

As stated above, applicants that have already submitted the results of biomonitoring test information over the past four years do not need to resubmit this data. Instead, indicate in question 2 the date you submitted each report and provide a summary of the test results for each report. Include in this summary the following information: the outfall number, toxicity testing method(s) used, and a summary of the results from the test (e.g., 100% survival in 40% effluent).

3. Toxicity Reduction Evaluation

A Toxicity Reduction Evaluation (TRE) is a site-specific study conducted in a stepwise process designed to identify the causative agents of effluent toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity. If the treatment works is conducting a TRE as part of a NPDES permit requirement or enforcement order, then you only need to provide the date of the last progress report concerning the TRE in the area reserved for details of the TRE. If the treatment works is conducting a TRE that is not required by the NPDES permit or an enforcement order, please provide the reasons for conducting the TRE and the current progress of the TRE.

4. Individual Test Data

Complete question 4 for each test conducted in the last four years for which data has not been submitted. If the treatment works is conducting WET tests and reporting its results in accordance with a NPDES permit requirement, then the treatment works may note the dates the tests were submitted in question 2 and need not fill out the information requested in question 4 (unless otherwise required by the permit writer). Page 2 of Part C (question 4) should be copied and given to testing laboratories as appropriate.

Facility Data.

- i. Provide the outfall number that was tested. This outfall number must correlate to the outfall numbers listed in question 14 of Part A.
- ii. If the facility uses dechlorination, indicate whether the sample was taken before or after dechlorination. If the facility does not dechlorinate, check the "Not Applicable" box.
- iii. Provide the dilute effluent sample ratio, as specified in the current NPDES permit, or as specified by the permit writer for a specific test.
- iv. Provide the sample number assigned by the testing laboratory.
- v. Provide the date the facility collected the sample for the specific test.
- vi. Provide the date the sample was received by the testing laboratory.
- vii. Provide the temperature of the sample when it was received by the testing laboratory, in degrees Celsius.
- a. Organism Data. For both test species, provide the age of the organism when the test was begun, the reference toxicant, the reference LC₅₀, and the date on which the reference toxicant was run.
- b. Sample Data. Provide the results of testing performed on the sample for each specified parameter.
- c. Data. Provide the results of the testing, and include and comments as necessary.

Attach any chronic toxicity test results for which data has not been submitted to this form, and submit them with the application packet.

5. Acute and Chronic Test Results.

The applicant must only complete question 5 if the permit writer has required acute and/or chronic WET testing based on TDS sample results. If such testing is not required, question 5 may be left blank. In addition to submitting the laboratory report, the facility must provide the information requested in question 5. If the facility wishes to do pass/fail testing based on TDS sample results, it is acceptable. Question 4 must be completed, as well as part c of question 5.

- a. For acute toxicity tests, provide the LC₅₀ (Lethal Concentration to 50 percent) of the test. "LC₅₀" is the effluent (or toxicant) concentration estimated to be lethal to 50 percent of the test organisms during a specific period. Provide the NOAEC (No Observed Adverse Effect Concentration). "NOAEC" is the highest measured concentration of an effluent (or a toxicant) at which no significant adverse effects are observed on the test organisms at a specific time of observation. Provide the 95% confidence interval, control percent survival, and any other test results requested by the permit writer in the space provided. For all parameters except the control percent survival, results may either be provided as percentages or as concentrations.
- b. For chronic toxicity tests, provide data at the most sensitive endpoint. While this is generally expressed as a NOAEC, it may be expressed as an Inhibition Concentration (e.g., "IC₂₅"--Inhibition Concentration to 25 percent). "IC₂₅" is the effluent (or toxicant) concentration estimated to cause a 25 percent reduction in reproduction, fecundity, growth, or other non-quantal biological measurements. Provide the 95% confidence interval, control percent survival, and any other test results requested by the permit writer in the space provided. For all parameters except the control percent survival, results may either be provided as percentages or as concentrations.
- c. Provide the requested ion concentrations in the test sample (100% effluent).